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Antibody Binding to the AAV Capsid and Modifying the Cytotropism Thereof and a Method for Targeted Gene Transfer

The present invention relates to antibodies or fragments thereof, which bind to the capsid of adeno-associated viruses (AAV) so as to prevent the binding of the virus to the virus receptor of the original target cell. This (These) antibody(ies) or the fragment(s) thereof may also serve as adaptors for fusing with the desired receptor ligands. After binding such an antibody to the AAV capsid, an AAV is obtained which has a modified tropism, i.e. can bind to a new target cell - depending on the fused ligand. Thus, the present invention also relates to AAV vectors to the capsid of which the antibody according to the invention or the fragment thereof is bound. These vectors may be used for targeted gene transfer. Finally, the present invention relates to a method for the targeted gene transfer using this AAV vector.

The first occurrence of a viral infection is the binding of the virus to the host cell surface. The cell surface molecules to which the viruses bind are referred to as virus receptors, whereas the viral proteins which are involved in this binding are designated as viral attachment proteins (VAP = viral cell attachment protein) or viral ligands. Studying the interaction between virus receptors and viral ligands has attracted heightened interest in the past few years, since based on the knowledge of this interaction it is not only possible to gather important information on the prevention of viral infections but such knowledge can, under certain circumstances, also be used for a well-calculated manipulation of the infection with viral vectors.

Identification of a greater number of viral receptors revealed a number of common features with the infection of

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various viruses. For example, different viruses use members
of the same family of cell surface molecules as viral
receptors, such as members of the IgG superfamily, the
multimembrane spanning transporter, integrins or growth
receptors. Furthermore, it turned out in some cases that the
same viruses cannot only use several receptor molecules but
that for a successful infection of the target cell the
interaction with several receptors is also required. In order
to understand the infection occurrence and optionally be able
to manipulate it, it is therefore above all necessary to know
the viral attachment proteins and if possible the attachment
sequences. The erythrocyte P antigen was described within the
parvovirus family as a receptor for the autonomous parvovirus
B19 (Brown et al., Science 262, pp. 114-117, 1993), and
heparane sulfate proteoglycan was determined for the helper-
dependent AAV-2 as a cellular receptor molecule (Summerford
and Samulski, J. Virol. 72 (2), pp. 1438-1445, 1998).

Viral vectors, e.g. retroviral vectors, adenoviral vectors or
vectors derived from adeno-associated viruses have preferably
been used for a gene transfer to date. A drawback of the
methods used thus far consists in that it has hardly been
possible to date to modify the virus such that (only) the
desired target cell is transduced (targeted gene transfer)
and the undesired transduction of non-target cells is
avoided. An extension or modification of the target cell
spectrum (i.e. tropism) could not only increase the
efficiency of the gene transfer but also create both *ex vivo*
and *in vivo* access to hardly transducible cell types. Thus,
the development of gene transfer methods as selective as
possible is gaining significance. In this connection,
targeted gene transfer becomes more and more important along
with the elements of intracellular specificity which can be
achieved e.g. by controlling the gene expression using
tissue-specific or cell-specific promoters.

The vectors used for a gene transfer thus far also comprise

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the AAV-based vectors. AAV is a human parvovirus which consists of a non-coated icosahedral capsid having a single-stranded DNA genome (about 4.6 kb). It has a wide host range and is capable of integrating its genome into a preferred site of the host cell genome if no helper virus is present (Kotin *et al.*, PNAS USA 87(6), pp. 2211-2215, 1990). Superinfection with a helper virus (e.g. adenovirus or herpes virus) mobilizes the latent AAV and induces an amplification of the AAV genome. About 70 % of the population have antibodies against AAV for which, however, no pathogenic properties are known. The vectors derived from AAV only consist of the two 145 bp long terminal repetitions which carry the signal in cis for replication, packaging and integration. Up to about 4.5 kb foreign DNA can be inserted between these two elements. Packaging into recombinant viral vectors (rAAV) requires in trans the rep and cap genes and a helper virus. Although several improved methods for the vector production have been published in the past few years, the relatively costly and time-consuming production of AAV vectors is still one of the main impediments for a wider application of this vector system. Recently, a decisive improvement and simplification of the production method could, however, be reached with the rAAV production without helper virus (Xiao *et al.*, J. Virol. 72(3), pp. 2224-2232, 1998).

AAV vectors combine a number of advantages: They contain no viral genes, have stable capsids and a wide host range and are capable of infecting both proliferating cells and resting cells. In particular, they permit long-term expression of introduced genes into the differentiated tissues, e.g. muscles, brain and retina, without notable immune response of the host.

The drawbacks of using AAV vectors for the gene transfer are however *inter alia* that although AAV has a wide host range, some cell types, e.g. hematopoietic stem cells and dendritic

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cells, can be transduced only with unsatisfactory results. In
addition, the possibility of selective gene transfer with AAV
would, of course, be fundamentally desired for *in vivo*
applications. However, there is hardly any knowledge of the
determinants of the cell and tissue tropism of AAV, such as
of viral attachment proteins or attachment sequences, and
thus there is presently no possibility of manipulating AAV as
regards a selective gene transfer.

Therefore, it is the object of the present invention to
provide AAV vectors by means of which a selective gene
transfer can be obtained.

This object is achieved by the subject matters defined in the
claims.

Monoclonal antibodies were produced in the present invention,
which are directed against the binding sites (ligand
sequences) of the AAV capsid proteins. It could be shown that
after binding of these antibodies to AAV the original target
cell was not infected and the binding of AAV to the viral
receptor of the original target cell was blocked. Moreover,
the binding properties of AAV capsids could be modified in
that based on the above-mentioned antibodies fusion
polypeptides (e.g. Fab fragments of the monoclonal antibody
linked with new ligand sequences) or single-chain antibody
fusion proteins were produced, which were bound to the AAV
capsids. It could be shown that AAV vectors having such
capsids only transfected the desired target cell and thus are
suited for a selective gene transfer.

Thus, the present invention relates to a monoclonal antibody
which is characterized in that it binds to the capsid of an
adeno-associated virus (AAV) and prevents binding of the
virus to the virus receptor of the original target cell.

The term "capsid" used herein refers to the icosahedral

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protein coat which surrounds the AAV genome and is typically composed of the structural proteins VP1, VP2 and VP3.

The term "original target cell" used herein designates any cell to which the unmodified AAV binds.

AAV include the following types: AAV-1, AAV-2, AAV-3, AAV-4, AAV-5 and AAV-6. For the purpose of gene transfer the following groups are particularly suited: AAV-2, AAV-3, AAV-4, AAV-5 and AAV-6.

Prevention of the binding of the AAV to the virus receptor of the original target cell may be determined by several methods. Various radioactive and non-radioactive binding tests have been developed in the past few years to determine the binding of a virus to its target cell. These methods can be applied within the framework of the present invention. In most approaches ^{125}I- or ^{35}S-labeled virus particles are incubated with cells in radioactive tests and the radioactivity remaining with the cells is measured subsequently (Summerford, C. and Samulski, R.J. 1998, Journal of Virology 72, 1438-1445; Grubman, M.J. et al. 1985, Journal of Virology 56, 120-126; Abraham, G. et al. 1988, Journal of Virology 62, 2300-2306; Greve, J.M. et al. 1989, Cell 56, 839-847). Other radioactive binding tests are based on blot assays in which cell extracts are dotted onto a nitrocellulose membrane or are blotted according to SDS-PAGE and they are then incubated with radioactively labeled viruses. Following exposition bound viruses can be detected on an X-ray film (Bass, D.M. et al. 1991, Virology 183, 602-610; Roivainen, M. et al. 1994, Virology 302, 357-365). Fewer approaches were carried out with non-radioactive binding tests. For example, Mizukami et al. (Virology 217, 124-130, 1996) used biotinylated AAV in place of radioactively labeled viruses which were detected by means of labeled streptavidine. Herrmann et al. (Journal of Virology 69, 6797-6804, 1995) measure the binding of B-lymphotrophic

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papovaviruses to cell lines of B-lymphomas by means of a capsid protein-ELISA while Tresnan et al. (Virology 211, 123-132, 1995) determined the cells bound by empty capsids of the canine parvovirus by FACS analysis. By means of these binding tests it is possible to not only determine the direct binding of viruses to the cell but also examine the blocking of this binding by monoclonal antibodies (Mak). In this connection, cells are incubated with Mak before an incubation with radioactively labeled viruses takes place and the cell-bound radioactivity is measured (Roden, R.B.S. et al. 1994, Journal of Virology 68, 7570-7574; Shepley, M.P. et al. 1988, Proceedings of the National Academy of Sciences U.S.A. 85, 7743-7747; Bergelson, J.M. et al. 1992, Science 255, 1718-1720). Brown, K.E. et al. (Science 262, 114-117, 1993) determined by means of colony-forming units Maks which prevent the binding of the B19 parvovirus to the cell.

The monoclonal antibodies described in the present patent application are preferably isolated via another non-radioactive approach. It is described below by means of AAV-2, without the application being restricted thereto. The non-radioactive binding assay was developed especially for the isolation of monoclonal antibodies which inhibit the binding of AAV-2 to the cell. In this test, AAV-2 viruses were pre-incubated with hybridoma supernatants to then be incubated with cells. These cells with the viruses bound thereto were fixed and non-specific binding sites were blocked to then detect the bound viruses by means of capsid ELISAs. The capsid ELISA uses an AAV-2-binding biotinylated monoclonal antibody which can be detected by streptavidine peroxidase. When screening the hybridoma supernatants, a negative ELISA result was searched for. In case the searched monoclonal antibody is capable of binding to AAV-2 and of preventing the binding of the virus to the cell, no signal is obtained in the ELISA since only AAV-2 particles which bind to cells are detected.

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Non-binding of a virus results in non-infection. However, these two processes are not identical, since a virus may bind cells but does not have to be accepted into the cell at the same time, which would result in the infection thereof.

Methods of obtaining antibodies are known to a person skilled in the art. The production of monoclonal antibodies comprises e.g. as a first step the production of polyclonal antibodies using AAV capsid proteins or fragments thereof (e.g. synthetic peptides) with suitable ligand sequences, e.g. the peptides described in the examples or fragments thereof, as immunogen for immunizing suitable animals and the recovery of cells producing antibodies against the defined antigen, e.g. sensitized B lymphocytes. Then, e.g. cell hybrids are produced from antibody-producing cells and bone marrow tumor cells (myeloma cells) and cloned. Thereafter, a clone is selected which produces an antibody specific to the antigen used. This antibody is then produced. Examples of cells preparing the antibodies are spleen cells, lymph node cells, B lymphocytes, etc. Examples of animals which can be immunized for this purpose are mice, rats, horses, goats and rabbits. The myeloma cells can be obtained from mice, rats, humans or other sources. The cell fusion may be carried out e.g. by the generally known method of Köhler and Milstein. The hybridomas obtained by cell fusion are screened by means of the antigen according to the enzyme-antibody method or a similar method. Clones are obtained by means of the boundary dilution method. The resulting clones are implanted intraperitoneally into e.g. BALB/c mice, the ascites is removed from the mouse after 10 to 14 days, and the monoclonal antibody is purified by known methods (e.g. ammonium sulfate fractionation, PEG fractionation, ion exchange chromatography, gel chromatography or affinity chromatography).

The antibody obtained can be used directly or a fragment thereof can be employed. In this connection, the term

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"fragment" refers to all parts of the monoclonal antibody
(e.g. Fab, Fv or single chain Fv fragments) which have the
same epitope specificity as the complete antibody. The
production of such fragments is known to the person skilled
in the art.

In a particularly preferred embodiment, said monoclonal
antibody is an antibody originating from an animal (e.g.
mouse), a humanized antibody, a chimeric antibody, a human
antibody or a fragment thereof. Chimeric antibodies which are
similar to human antibodies or humanized antibodies have a
reduced potential antigenicity but their affinity over the
target is not lowered. The general production of chimeric and
humanized antibodies or of antibodies similar to human
antibodies was discussed in detail (see e.g. Queen et al.,
PNAS U.S.A. 86, pp. 10029, 1989; Verhoeyan et al., Science
239, p. 1534, 1988). Humanized immunoglobulins have variable
framework regions which originate substantially from a human
immunoglobulin (designated acceptor immunoglobulin) and the
complementarity of the determining regions which originate
substantially from non-human immunoglobulin (e.g. from mouse)
(designated donor immunoglobulin). The constant region(s)
originate(s), if available, also substantially from a human
immunoglobulin. When administered to human patients,
humanized (and human) antibodies offer a number of advantages
over antibodies from mice or other species: (a) the human
immune system should not regard the framework or the constant
region of the humanized antibody as foreign and therefore the
antibody response to such an injected antibody should be less
than that to a fully foreign mouse antibody or a partially
foreign chimeric antibody; (b) since the effector region of
the humanized antibody is human it might interact in a better
way with other parts of the human immune system, and (c)
injected humanized antibodies have a half life substantially
equivalent to that of naturally occurring human antibodies,
which permits administering smaller and less frequent doses
as compared to antibodies of other species. These advantages

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apply, of course, to human antibodies as well.

In another particularly preferred embodiment, the antibody according to the invention is an antibody or a fragment thereof which binds to the capsid of AAV-2, AAV-3, AAV-4, AAV-5, or AAV-6 and prevents the binding of the virus to the virus receptor of the original target cell.

Among the above described antibodies or fragments thereof particularly those are preferred which bind to the capsid proteins VP1, VP2 and VP3, in particular in the region of amino acids 449-475, 545-556 and 585-598 (based on VP1 of AAV-2).

Antibodies according to the invention may be obtained from the hybridoma cell lines deposited with DSMZ (German-Type Collection of Microorganisms and Cell Cultures), Mascherode Weg, Braunschweig under numbers ACC 2369 (is C24-B) and ACC 2370 (is C37-B) on August 19, 1998. They are directed against AAV-2.

The present invention also relates to the above described monoclonal antibodies which are additionally characterized in that they are fused with a desired receptor ligand and thus may serve for constructing AAV vectors with an extended or reduced host region – depending on the introduced new ligand sequence. As receptor ligands all ligands are suitable which effect extension or reduction and thus well-calculated modification of the host region. For this purpose ligands are also suitable which bind preferentially to receptors of malignant cells. For example, the following ligands are in consideration for this:
-     folate since the folate receptor is expressed increasingly in uterine carcinomas, lung and breast cancers as well as brain tumors,
-     fibroblast growth factor (FGF), since the high affinity FGF receptors can be found in malignant

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cell lines (e.g. SKOV 3.ip1, a human uterine carcinoma cell line) and primary cells (e.g. Kaposi's sarcoma cells) and proliferating endothelial cells in tumors,

- RGD peptide motives binding to $á_v$ integrins which are found almost exclusively on endothelial cells of angiogenic capillaries in tumors

(melanomas, carcinomas, sarcomas)
- epidermal growth factor (EGF)
- CD 19.

In addition to receptor ligands which are suitable for targeting tumor cells, ligands permitting the infection of target cells enabling the treatment of genetic diseases are also of great significance for those receptors. These are for example:

- anti-human secretory component Fab fragments. They bind to the polymeric immunoglobulin receptor (pIGR) for gene transfer in epithelial cells of the respiratory apparatus as a method of treating cystic fibrosis,
- asialoglycoprotein (ASGP) for gene transfer into the liver,
- erythropoietin for targeting hematopoietic cells which carry the erythropoietin receptor, e.g. for treating sickle-cell anemia.

Furthermore, there are monoclonal antibodies which in place of ligands bind specifically to certain receptors. They are also of great significance as potential fusion partners to the monoclonal antibodies according to the invention.

There are a number of different possibilities for the construction of these fused antibodies or fragments thereof. For example, an antibody fragment, e.g. a Fab fragment, may

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be linked directly with the ligand sequences binding to the receptor of the desired target cell. A large variety of reagents for chemical linkage, also referred to as cross-linker, have been developed in the past few decades. In general, these linking reagents which provide at least two reactive groups during the linkage can be divided into homobifunctional and heterobifunctional cross-linkers. The former have at least two identical reactive groups and permit one-step linkage whereas the latter have at least two different reactive groups and permit sequential conjugation of proteins. The most frequently used cross-linkers are homobifunctional and react with the primary amino groups of proteins. They comprise imido esters and NHS esters (N-hydroxysuccinimide). NHS esters are more stable and efficient than imido esters and react with primary as well as secondary amines to form an amide bond. Further homobifunctional cross-linkers are sulfhydryl reagents which react with thiol groups and other conjugation reagents which interact with other reactive groups (arginine-specific or carbonyl-specific cross-linkers) or show no selectivity (e.g. photoaffinity reagents). The above mentioned cross linkers link proteins via bridges permitting a differing distance of the proteins. The known method in which no bridges form is the carbodiimide method in which carboxyl groups are linked with primary amines via an amide bond. When linking reagents are selected, it is above all necessary to consider the reaction buffers and pH in which the cross-linker is active and the stability of the proteins to be linked in this medium. As an alternative, the binding sequence of the monoclonal antibody may also be cloned as a single-chain antibody. The approach developed for this purpose in the cloning of scFv consisted in the direct cloning of the antibody genes from hybridoma cell lines. In this connection, the variable regions of the light and heavy chains are amplified by means of polymerase chain reaction using antibody-specific oligonucleotide primers. (F. Breitling & S. Dübel, *Rekombinante Antikörper* [recombinant antibodies], Spektrum Akademischer Verlag

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Heidelberg, 1997). The cloned single-chain antibodies may be expressed e.g. on a phage surface within a phage library to thus examine their binding to AAV capsids e.g. by means of ELISA. Only those single-chain antibodies which show good binding are further used to express them e.g. in *E. coli*. By means of the expressed single-chain antibody purified e.g. via His-tags it is then possible to examine their capability regarding the competition of the binding of the virus to the receptor of the original target cell. Thereafter, the thus obtained single-chain antibodies may be fused with the desired ligand sequences or with a second single-chain antibody. The production of scFv with multivalent and multifunctional properties can in this case by achieved by various approaches. Some scFv show a natural multimerization potential, the variable domains of a scFv binding with the complementary domains of another scFv. Other scFv may be fused with one another by the fusion with a leucine zipper or an amphiphatic helix at the C terminus. A multifunctional approach relates to the fusion of an scFv with streptavidine. Biotinylated ligands, monoclonal antibodies or further scFv may then simply be conjugated with the scFv streptavidine. This simple conjugation to potential binding partners is also achieved by the introduction of C-terminal cysteines which form stable dimers via disulfide bridges. The direct fusing of two scFv results in a bivalent and bispecific diabody. Here, the VH domain of an scFv is linked via a short linker with the VL domain of the other scFv and the complementary VH-VL pair (Little *et al.*, Methods in Molecular Medicine, 555-622, Vol. 13: Molecular Diagnosis of Infectious Diseases, Humana Press Inc., Totowa, NJ). After the expression, e.g. in *E. coli*, such antibody-fusion proteins or bivalent antibodies may be used for the production of modified AAV vectors for the selective gene transfer.

Thus, the present invention also relates to an AAV vector, preferably based on AAV-2, AAV-3, AAV-4, AAV-5 or AAV-6, which is characterized in that the above described monoclonal

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antibodies according to the invention or fragments thereof are bound to its capsid which can no longer bind to the virus receptor of the original target cell but optionally to the virus receptor of a desired target cell. Such a vector permits the introduction of foreign DNA into a desired target cell. Thus, the present invention also relates to the above described vector which contains additionally a foreign DNA. It may be obtained e.g. by co-transfection of an AAV vector plasmid which carries a foreign DNA to be expressed between the inverted terminal repeats (ITR) of AAV with an AAV helper plasmid in a production cell line (e.g. 293T cells) and superinfection with a helper virus (e.g. Ad2, Ad5 or HSV-1) (figure 1a). As an alternative, the necessary functions of the helper virus may also be present on the AAV helper plasmid so as to simplify the production process (figure 1B). The foreign DNA to be expressed may contain a reporter gene or a gene of therapeutic interest. This foreign DNA should not exceed a maximum size of about 4.7 kilobases and may be selected by a person skilled in the art as desired. The recombinant AAV viruses (vectors) may be released by cell lysis from the transfected cells. A modification of the capsids with the described antibodies may be carried out in the cell lysate or after the purification of the vectors. The purification may be made by means of various methods with which the person skilled in the art is familiar.

The AAV vector is preferably modified such that it can bind target cells via different receptors. For example, tumor cells having receptors such as the folate receptor, the epidermal growth factor receptor and fibroblast growth factor receptor or receptors of hematopoietic cells such as the erythropoietin receptor, SCF receptor and CD 34 are in consideration as target cells.

The present invention also relates to a method of producing the AAV vector according to the invention which comprises the steps described in the above sections.

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The present invention also relates to a method for the targeted gene transfer, which is characterized in that the AAV vector according to the invention is used as a vehicle for the nucleic acid sequence to be introduced into the desired target cell. The nucleic acid sequence to be introduced may e.g. be controlled by an inducible and/or repressible cell type-specific promoter. The AAV vectors according to the invention may be introduced into a cell, a tissue, organ, a patient or an animal by a number of methods, e.g. by *ex vivo* incubation of the purified AAV vectors with the desired target cells (e.g. Maas *et al.*, Human Gene Therapy 9, 1049-1059 (1998), Zhou *et al.*, Gene Therapy 3, 223-229 (1996), Ponnazhagan *et al.*, Journal of Virology 71, 8262-8267, (1997)) or by direct injection into a target tissue (e.g. Xiao *et al.*, Journal of Virology 70, 8098-8108, Flannery *et al.*, Proc. Natl. Acad. Sci. U.S.A. 94, 6916-6921 (1997), During *et al.*, Gene Therapy 5, 50-58 (1998)).

Finally, based on the present invention it is also possible to obtain cells and transgenic animals (i.e. mammals) which are transgenic with respect to the nucleic acid sequence introduced by means of the AAV vector according to the invention. Methods for the production of such transgenic animals may be found e.g. in WO 91/08216.

The present invention is described in more detail by means of the figure:

Figure 1 A, B: Diagram of methods for the production of AAV vectors.
Figure 2:   Diagram of a test for isolating antibodies which block the binding of the virus to the cell.
Figure 3: Retargeting of AAV-2.

The below examples explain the invention.

Example 1:   Production of antibodies directed against the

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ligand sequences

For the production of antibodies, Balb-c mice were immunized repeatedly with synthetic peptides and with empty AAV-2 capsids. The four below peptides were synthesized due to AAV-2 capsid gene sequences in which AAV-2 and AAV-3 are different. The peptides had the following sequence:

> AAV-2-1: GPPPPKPAERHKDDSC
>
> AAV-2-2: SRTNTPSGTTTQSRLQFSQAGASDIRDQSC
>
> AAV-2-3: QSGVLIFGKQGSEKTNVDIEKC
>
> AAV-2-4: SVSTNLQRGNRQAATADVNTQC

The two viruses AAV-2 and AAV-3 have a different cytotropism although their capsid gene sequences differ slightly in only four domains. Since peptides only represent linear epitopes but the ligand sequence on the viral capsid may also represent a conformative epitope, booster was additionally carried out with empty AAV-2 capsids.

100 μg peptide or 30 μg empty (containing no DNA) assembled AAV-2 capsids in 0.1 ml PBS and 0.1 ml complete or incomplete Freund's adjuvant (icFA) were used per immunization:

Day 0: 1st immunization (peptide + complete Freund's adjuvant)

Day 24: 2nd immunization (peptide + icFA)

Day 41: 3rd immunization (capsids + icFA)

Day 132: 4th immunization (capsids + icFA)

Day 145:   fusion.

Having concluded the immunization, the spleen of a mouse was removed and the spleen cells isolated therefrom were fused with Ag8 cells. The resulting clones separate antibodies to the medium which was then tested with various assays for its properties. First, all hybridoma supernatants were examined as to their capability of neutralizing. In this neutralization test based on GFP (green fluorescent protein)

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screening of the monoclonal antibodies took also place. Cells were seeded in 96-well plates and incubated with a mixture of AAV-2 GFP particles (MOI 10) and hybridoma supernatants the next day. 20 hours after the infection, the cells were examined as to the expression of GFP under U.V. light. Hybridoma supernatants which have a neutralizing effect, i.e. those which prevent the expression of GFP, could then be examined as to their ability of preventing the binding of AAV-2 to the cellular receptor. This non-radioactive binding test was developed especially for this task (see figure 2). For this purpose, hybridoma supernatants were first incubated with AAV-2 before they were placed on cells, e.g. HeLa cells. The AAV-2 particles binding to the cell were fixed. Hybridoma supernatants which bind to AAV-2 and prevent the binding of the capsid to the cellular receptor, prevent the binding to the cells. After a blocking step, the viruses bound to the cells are detected with the monoclonal A20 antibody which detects assembled AAV-2 capsids (Wistuba et al., Journal of Virology 71, p. 1341-1352, 1997). The thus characterized clones which produce neutralizing and binding-inhibiting antibodies were then isolated to obtain the monoclonal antibodies (Mak). The resulting Maks were again examined as to their properties. In addition to the just described assay they were also examined as to their behavior in immunofluorescences of Ad-5 (negative control) and Ad-5/AAV-2-infected cells, Western blots of Ad/AAV-2 HeLa extracts and in the AAV-2 ELISA. Having analyzed these data and determined the subclasses the two hybridomas C24-B and C37-B were chosen. The following table shows their properties.

Assay	C24-B	C37-B
Neutralization test	+	+
Binding test	+	+
Western blot analysis	–	–
Immunofluorescence adeno/AAV-2-infected cells	+	+
Adeno-infected cells	–	–

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AAV-2 ELISA	+	+
Immunoglobulin subclass	IgG1	IgG1

The two hybridomas were deposited with the DMSZ in Braunschweig on August 19, 1998 under the following numbers:

     C24-B: ACC 2369

     C37-B: ACC 2370

Example 2:   Production of fused single-chain antibodies

Single-chain antibodies (scFv) are produced from both hybridomas, C34-B and C37-B. After isolating DNA, mRNA and the subsequent synthesis of cDNA, it was possible by means of oligonucleotide primers whose sequence is published in Breitling et al., Methods in Molecular Medicine, pp. 581-592, Vol. 13: Molecular Diagnosis of Infectious Diseases, Humana Press Inc., Totowa, NJ, to isolate in the polymerase chain reaction (PCR) the variable domains of the light (VL) and the heavy chain (HV). They were cloned according to standard methods into the expression plasmid pHOG21 (Kipriyanov, S.M. et al., 1997, J. of Immunol. Methods 200, pp. 69-77).

Diabodies are then prepared in cooperation with the study group of Professor Dr. M. Little of Deutsches Krebsforschungszentrum. These diabodies are bivalent and bispecific, since they consist of two scFv fused with each other. Here, the VH domain of one antibody (C24-B or C37-B) is linked via a short linker having the sequence "AKTTPKLGG" (peptide linker which bridges the about 3.5 nm between the C-terminus of one domain and the N-terminus of the other domain (Kipriyanov, S.M. et al., Int. J. Cancer 77, pp. 763-772, 1998) with the VL domain of another antibody (anti-CD19) and vice versa. General references to the production of diabodies are "Little et al., Methods in Molecular Medicine 555-580, Vol. 13: Molecular Diagnosis of Infectious Diseases, Humana Press Inc., Totowa, NJ". As a result, a product forms which

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has two antigen binding domains which lie on opposite sides
of the complex.

The above diabodies will be composed of the svFv of C24-B or
C37-B and scFv of antibodies binding to B cells. However,
there is the possibility of introducing all scFv directed
against cell receptors into these diabodies.

Example 3:   Chemical linkage of Fab fragments

Fab fragments of C24-B and C37-B were isolated according to
standard methods. They are then chemically linked with IgGs
which are directed against EGF and FGF receptors or with
folate, the ligand of the folate receptor. The conjugation is
made by means of SPDP(3-(2-pyridildithio)propionic acid N-
hydroxysuccinimide ester). The resulting complexes are
purified over HPLC and can then be used to modify the
cytotropism of AAV vectors. For this purpose, AAV vectors are
incubated with the purified Fab-IgG ligand complexes. The
complexes not bound to the AAV vectors are separated (e.g.)
by centrifugation through a sugar cushion.

In particular, AAV-2 vectors are used with the luciferase or
LacZ reporter genes to obtain a quantitative and qualitative
information on the obtained expression efficiency. However,
it is, of course, possible to use all available AAV vectors.
These vectors are then incubated with the Fab-IgG/ligand
complexes.

Example 4:   Change of the tropism of rAAV-2

The diabodies described in Example 2 and the Fab-IgG/ligand
conjugates described in Example 3 can then be used for
changing the tropism of rAAV-2.

Two general application possibilities offer themselves. On
the one hand, the Fab-IgG/ligand conjugates are used to test

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the principle that rAAV-2 cells can be infected via a new receptor. Following the linkage of Fab fragments to the ligand folate, for example, and subsequent incubation with rAAV-2, it is shown that cells (e.g. HeLa, KB) which overexpress the folate receptor can be infected. This infection cannot be prevented by an excess of heparin which inhibits the natural AAV-2 infection but by the presence of free Fab fragments or an excess of folate in the medium. By means of cell lines which express e.g. the EGF receptor (MDA, MB468 or U118) retargeting to the EGFR can be shown by means of the Fab-anti-EGFR-IgG conjugate in analogy with the above described example (see figure 3).

However, a change of the tropism is also possible via the described diabodies. They are used to make cells which can poorly be infected for AAV-2 (e.g. the cell lines Raji [human Burkitt lymphoma cell line], 9023 and 9050 [human lymphoblastoid cell lines]; Maass, G. *et al.* Human Gene Therapy **9**, 1049-1059, 1998) more accessible to an AAV-2 infection. For this purpose, the diabodies are incubated with rAAV-2, the diabodies not bound to the AAV capsids are separated and then placed on the above-mentioned cells. Thus, an efficient infection of the lymphoma cell lines which can hardly be infected by non-modified AAV-2 is now possible.